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Intermediate Filaments: Vimentin Moves in

Dispatch

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Vimentin intermediate filaments move bi-directionally along microtubules in the cell. Recent work has identified the microtubule motor cytoplasmic dynein as the missing inward-directed motor that drives this movement.

Intermediate filaments are one of the three major cyto-skeletal networks in eukaryotic cells. Several distinct types exist, assembled from different subunits and with characteristic expression patterns. Until recently, intermediate filaments had been considered to be relatively stable, and were proposed solely to provide cellular integrity and resistance against mechanical stresses [1]. In recent years, however, live cell imaging has made it clear that these filaments are highly dynamic in their assembly and organisation ([2–6], for example).

The dynamics of vimentin filaments are probably the best characterised to date. The vimentin network has a radial organisation, extending outwards from the cell centre. This localisation partially overlaps with that of the microtubule array, and many previous studies have suggested that the two filament systems interact (summarised in [2,3]). Live imaging of cells expressing a GFP–vimentin fusion protein has revealed that the vimentin network is motile, with filaments constantly changing their shape [2,5]. The motile properties of vimentin filaments are particularly evident in spreading cells, where three different structural forms of vimentin are involved in the assembly of the vimentin network [3]. Small dots or particles elongate into short fibrils, termed ‘squiggles’, which themselves are converted into longer vimentin filaments over time. The various vimentin structures have all been shown to move along microtubules, and research has identified two candidate motors for this motility: conventional kinesin, and more recently, cytoplasmic dynein [3,5,7,8].

Why are two motors needed? The key to this question lies with the microtubules, which are polar structures with rapidly growing ‘plus’ ends that are generally located at the cell periphery, and slow-growing ‘minus’ ends usually associated with the centrosome. Conventional kinesin, and many kinesin-like proteins, move towards microtubule plus ends, whilst cytoplasmic dynein [9], and some kinesin-like proteins, move in the opposite direction. The fact that all forms of vimentin filaments in living cells move along microtubules both towards and away from the cell centre [3,5] suggests that two motors of opposite polarity are active in their movement.

This idea is supported by the observation that micro-injection of cultured cells with antibodies to conventional kinesin not only prevented the extension of the vimentin network to the edge of the cell [3], but also caused the retraction of the vimentin network into a tight perinuclear aggregate [3,7], presumably because the minus-end-directed motor was still active under these conditions (Figure 1). As vimentin particles and fibrils can be labelled with antibodies to conventional kinesin [3], and *in vitro* binding assays have demonstrated an association between conventional kinesin and vimentin [8], it is highly likely that kinesin is the plus-end-directed motor which transports vimentin filaments.

Helfand and colleagues [5] have now shown that the inward movement of vimentin filaments in the cell is dependent upon cytoplasmic dynein and its regulatory complex dynactin [9]. Immunofluorescence revealed that cytoplasmic dynein and dynactin subunits partially colocalised with vimentin particles, squiggles and filaments, most obviously at intersections between vimentin filaments and microtubules, as would be expected if the cytoplasmic dynein–dynactin complex transports vimentin along microtubules. Furthermore, cytoplasmic dynein and dynactin were also present in an intermediate filament-rich cytoskeletal preparation.

Helfand *et al.* [5] also made use of a powerful tool for assessing the function of cytoplasmic dynein — overexpression of dynamitin, which causes dissociation of the dynactin complex [10] and so prevents cytoplasmic dynein from binding to its cargo [10,11]. Live imaging of co-transfected cells expressing GFP-tagged vimentin and dynamitin showed a reduction in the amount and speed of inwardly directed GFP–vimentin motility in comparison to control cells. As a dramatic consequence, vimentin filaments were cleared from the perinuclear region of the cell and accumulated at the cell periphery. Cytoplasmic dynein–dynactin was absent from these peripheral vimentin filaments, as judged by immunofluorescence, whilst conventional kinesin was still associated, suggesting that the vimentin structures were transported to the edge of the cell by kinesin. Correspondingly, there was less cytoplasmic dynein–dynactin in the intermediate filament-rich fraction after dynamitin overexpression, whilst kinesin levels were unaffected.

Interestingly, exogenous dynamitin was found in the pellet [5], raising the possibility that dynamitin may provide the link between the dynactin complex and the vimentin filament cargo, as it does with BicD2 in the Golgi apparatus [12], and ZW10 at the kinetchore [13]. To check the effectiveness of dynamitin overexpression in these experiments, Golgi apparatus scattering was monitored, which is a classic measure of cytoplasmic dynein inhibition [11]. This assay was particularly interesting as it has been proposed that

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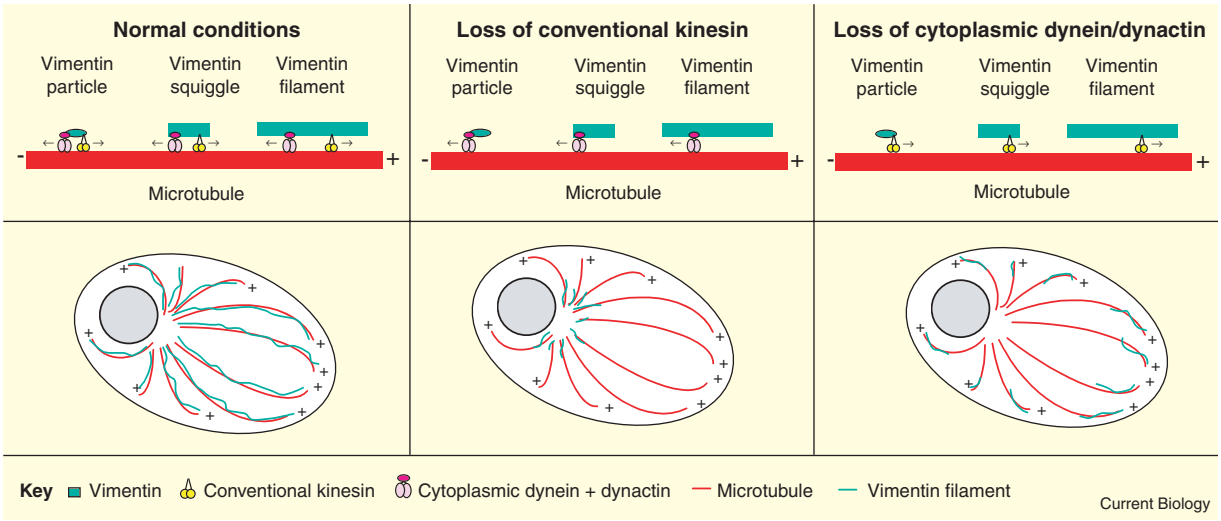

Figure 1. A model for vimentin movement.

All forms of vimentin (see text and [3,5]) move bi-directionally along microtubules by the action of kinesin and cytoplasmic dynein–dynactin, as shown by the effects of disrupting both motor activities in turn. Microtubule polarity is denoted by + and – signs.

the Golgi apparatus can interact directly with vimentin [14], and so it was possible that the alterations in Golgi positioning were caused partly by the changing vimentin distribution. This seems unlikely, however, as the scattered Golgi elements did not colocalise at all with peripheral vimentin filaments [5].

Together, these findings support the hypothesis that the extended vimentin intermediate filament network is maintained by a balance between the activities of conventional kinesin and cytoplasmic dynein (Figure 1). In addition, vimentin subunits and filaments can be transported in both directions for localised assembly in specific regions of the cell according to the cell's mechanical needs. One slight fly in the ointment is that this model does not explain why vimentin filaments collapse into the cell centre when microtubules are depolymerised [2] or when antibodies to tubulin are microinjected [15], raising the possibility that other interactions, perhaps with the actin cytoskeleton, may be involved. This might also explain the remaining inward vimentin filament motility seen in dynamitin overexpressing cells, although in this case the movement could conceivably be driven by a minus end-directed kinesin-like protein [5].

Does this model hold true for other types of intermediate filament? A similar hypothesis has, in fact, already been proposed for neurofilaments. Neurofilaments move bi-directionally *in vivo* and *in vitro* (reviewed in [16]) and, as with vimentin, plus end-directed transport of neurofilaments is mediated in part by conventional kinesin or a kinesin-like protein [6,16–18], whilst minus end-directed transport *in vitro* was inhibited by antibodies against dynein intermediate chain [17]. Furthermore, native neurofilaments associate with cytoplasmic dynein and dynactin subunits both biochemically and at the ultrastructural level [17].

Cytokeratin filaments are also motile, although they move much more slowly than vimentin filaments, and their inward movement is not affected by inhibiting cytoplasmic dynein function [4]. As cytokeratin filament assembly and movement in *Xenopus* egg extracts involves interaction between cytokeratin and actin filaments [19], this takes us back to the question posed above of whether there are also interactions between intermediate filaments and actin filaments within somatic cells. Indeed, data from a wide variety of systems suggest that all three filament types interact dynamically in complex ways.

What use are intermediate filament motility and dynamics to the cell? In small cells such as fibroblasts, one can speculate that such flexibility enables the cell to move and reorganise its cytoplasm and organelles when needed. For larger cells, especially neurons, microtubule motors provide the means for delivering intermediate filament components from their site of synthesis to the furthest regions of the cell — possibly more than a metre away. It is therefore perhaps not surprising that a failure of this delivery system may be of particular importance in neurodegenerative diseases, such as amyotrophic lateral sclerosis, where neurofilament aggregates are observed in the cell body and axon of motor neurons [20]. The specific disruption of one or both of the molecular motors involved in intermediate filament organisation may cause, or exacerbate, this aggregated phenotype, with serious effects on the cell.

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